# Corso di Dottorato di Ricerca in Alimenti e Salute Umana

Ciclo 33°

Titolo della tesi:

# INFLUENCE OF THE DIET ON LIPOFILLING RESORPTION RATE

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*Non andartene docile in quella buona notte.*
*Infuriati, infuriati contro il morire della luce.*
*(Dylan Thomas)*

*Dedico questo lavoro alla memoria del mio amico Andrea Simone Lerussi.*

*Andrea, mi sarebbe piaciuto festeggiare con te questo traguardo, purtroppo dovremo festeggiare in due dimensioni diverse.*

*I tuoi genitori ti hanno descritto come un guerriero, penso che tutti noi dovremmo essere dei guerrieri nella ricerca contro il cancro.*

# INFLUENCE OF THE DIET ON LIPOFILLING RESORPTION RATE

# Contents

# 1. Background

## 1.1 Breast Cancer, Obesity and Quality of Life

According to "Associazione italiana registri tumori" data, in 2015 in Italy 692.955 women had a diagnosis of breast cancer. Compared to 2010, this number has grown by 19% (1). Breast cancer survivals report that cancer has had a great impact on their quality of life (QoL) due to the treatments they had undergone. QoL of these patients has become increasingly important because of their high survival rate and prolonged life expectancy (2).

Lifestyle behaviours are well-established risk factors for both the development of breast cancer and the poorer long-term prognosis (3). Furthermore, obesity is an additional clinical challenge that influences mortality for breast cancer survivors. Several studies have shown that this disease is associated with a 30% high risk of mortality in all types of breast cancer (4, 5). As shown by Swisher et al, survivors of triple-negative breast cancer with BMI >25 which participated in a specially created program (Get Fit for the Fight®, controlled clinical trial NCT01498536) that comprised moderate-intensity aerobic exercise (150 min per week, for 12 weeks) and diet counselling reported better results in weight loss and QoL compared to control group.

Moreover, obesity negatively affects relationships and self-confidence of women who survived breast cancer, and it is a risk factor for lower self-esteem and greater sexual dissatisfaction. These weight concerns affect greatly the QoL of patients.

Regular exercise and healthy eating are routinely recommended for breast cancer survivors, and different studies have shown how they provide benefits both in the QoL of the patients and in decreasing inflammation. BMI has been associated with favourable changes in

leptin and adiponectin which may reflect a change in adiposity with intervention (6, 7). Weight loss can show promising results on reproductive, metabolic and psychological level. Several studies have demonstrated how exercise and healthy eating should be encouraged as a part of a cancer survivorship program, as described by Swisher et al (4): the Get Fit for the Fight® program consisted of supervised, moderate-intensity aerobic exercise three times per week at the exercise facility and two unsupervised sessions per week at home. Participants utilized a variety of exercise equipment (stationary bicycle, treadmill, elliptical trainer, etc.) for a total of 30 min of aerobic exercise. The goal of the program was to complete 150 min per week of moderate-intensity aerobic exercise, corresponding to 60–75 % of peak heart rate achieved on the exercise test. Dietary counselling consisted of two individual sessions with a specialist in nutrition for cancer patients. Participants completed a 3-day diet record during baseline testing before meeting individually with the dietitian. She then reviewed the diet record and helped participants set goals for changes, such as decreasing portion sizes, substituting lower-calorie options, and increasing fruit and vegetable intake. The goal of diet counselling was to decrease dietary fat caloric intake by 200 kcal per week.

## 1.2 Breast Reconstruction

The diagnosis of breast cancer is hard to bear for women, because the consequences of the mastectomy surgery affect their femininity and influence how much they feel comfortable in their daily life. Breast is a sexual organ which characterizes the body of women from the puberty. Feminine clothes and underwear are shaped taking into account a thorax with breast. Moreover, currently the female body is even more expected to fulfil certain aspects determined from the images on social media and the internet, often unrealistic, and this could be very stressful for a woman with such a diagnosis. As a consequence, patients could have difficulties to find

a new role in a society which has become dystopic regarding women's bodies, beside facing demanding therapies and surgeries.

Fortunately, in the last decades QoL for breast cancer survivors has improved due to the development of different techniques offered for both breast cancer management and breast reconstruction.
As Nahabedian states in his work "Oncoplastic Surgery of the Breast", prior of the era of William Halsted the diagnosis of breast cancer was often associated with few options for breast cancer management and poor patient survival. With Halsted radical mastectomy, morbidity and mortality of breast cancer diminished, despite the consequent deformity of the chest (8). Later, association between radiotherapy and simple mastectomy, performed preserving the pectoral major muscle, increased the availability of less aggressive surgical techniques (8). Nowadays, we know that skin and nipple areola complex can be spared without risk of local recurrence or decrease of the survival pattern, along with selected axillary dissection due to the routinely performed sentinel node biopsy technique.
Although there has been a significant improvement in techniques developed to perform even less invasive mastectomies, all of them resulted in disfigurement of the breast (Table 1-1). Therefore, in order to reduce the deformity of the chest, breast reconstruction has begun to become popular and widely studied (8).

*Table 1-1: Development of mastectomy techniques during the years [8]*

| **Chronological history of operations related to total mastectomy** | | |
|---|---|---|
| **Author** | Year | Treatment |
| **Halsted** | 1890 | Radical mastectomy |
| **Patey** | 1948 | Modified radical mastectomy |
| **McWhirter** | 1948 | Simple mastectomy and radiotherapy |
| **Toth** | 1991 | Skin-sparing mastectomy |

| | | |
|---|---|---|
| **Noguchi** | 1996 | Sentinel lymph node biopsy |
| **VerHeyden** | 1998 | Subcutaneous mastectomy (malignant disease) |
| **Chronological history of operations related to partial mastectomy** | | |
| **Crile** | 1973 | Partial mastectomy |
| **Montague** | 1978 | Breast conservation therapy |
| **Veronesi** | 1994 | Segmental parenchymal excision |
| **Gabka** | 1997 | Oncoplastic surgery |
| **Clough** | 1998 | Reduction mastopexy lumpectomy |
| **Amanti** | 2002 | Periareolar parenchymal excision |
| **Anderson** | 2005 | Parallelogram excision patterns |

The techniques used for breast reconstruction consider a replacement both in volume and in shape, and are generally divided in heterologous and autologous techniques, which allow to find the best solution for every woman and breast (Table 1-2).
Heterologous breast reconstruction procedures with breast implant or expander have widened their indication due to the development of acellular dermal matrices, that have permitted to extend the reconstruction with prosthesis also to women who have ptotic breast or thin skin, decreasing post-surgical pain and the damage to the muscles.
Autologous breast reconstruction techniques have extended from classic Latissimus Dorsi flap (first described by Igino Tansini in 1906) and TRAM or DIEP flaps (respectively, Transverse Rectus

Abdominis Muscle flap and Deep Inferior Epigastric Perforator flap) to intercostal artery perforator flaps, gluteal flaps, gracilis flap and lipostructure (as known as lipofilling or fat graft).

*Table 1-2: Development of breast reconstruction techniques during the years [8]*

| **Chronological history of operations related to breast reconstruction** | | |
|---|---|---|
| **Author** | Year | Technique |
| **Berson** | 1944 | Derma-fat grafts |
| **Longacre** | 1953 | Local flaps |
| **Snyderman** | 1969 | Prosthetic devices |
| **Arnold** | 1976 | Omentum and prosthetic |
| **Schneider** | 1977 | Latissimus dorsi |
| **Hartrampf** | 1982 | TRAM flap |
| **Argenta** | 1984 | Tissue expansion |
| **Grotting** | 1989 | Free TRAM flap |
| **Allen** | 1994 | Perforator flaps |

Among these, fat grafting (lipofilling) is a widely used practice refined by plastic surgeons (9). Studying the history of fat graft, it is recognised that Czerny in 1895 performed one of the first attempts of breast reconstruction with fat graft, transplanting a lipoma excised from the dorsum to a defect originated from a wide mammary gland removal. Later in 1931, Lexer tried a breast reconstruction with axillar adipose tissue. May, in 1941, described a similar attempt including the fascia in the fat graft to increase the vascularization. Then, several other authors have attempted analogous procedures for breast augmentation (10).
Illouz succeeded in a turning point in fat graft transfer technique, as he understood that fat could be suctioned by means of a cannula instead of being just surgical excised. Then, in 1991, Coleman described his technique called Lipostructuret® at the Congress of Lipoplasty Society of North America in Seattle, a real milestone in fat harvesting and transfer. He comprehended that fat should be

manipulated without causing trauma to the cells and described a protocol for fat manipulation (10).

## 1.3 The Lipofilling Technique

As described before, from Bircoll in 1987 to Coleman in 1995 many authors have contributed to the development of the technique of fat grafting (11). The procedure consists of three steps:

- harvesting the fat by means of liposuction
- processing the fat
- re-injection of the purified fat or lipofilling (Figure 1-1).

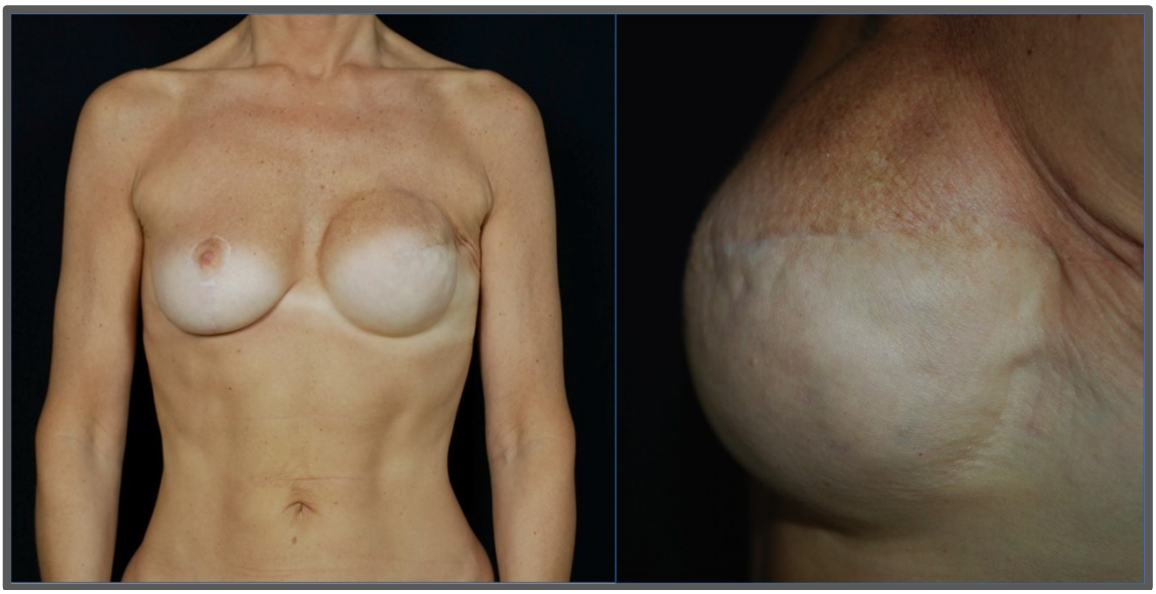
*Figure 1-1: Breast lipofilling*

The success of this technique is due to the properties of the adipose tissue, which is an easily accessible source that can be collected by

procedures relatively simple, safe and repeatable with minimal morbidity. Adipose tissue is available in the majority of patients and this allows autologous grafts without immunogenic drawbacks. Traditionally, liposuction sites are those that have always been subjected to liposuction for cosmetic reasons, such as abdomen, flanks, hips (12).
Fat can be transplanted in a wide range on anatomical areas with very different purposes; for instance, Vaienti described how lipofilling represents a valuable and versatile option in the treatment of painful neuromas of the upper limb and is also useful in pain syndromes of neuropathic origin treatment (12-15).

Analysing just the breast related field, fat graft is employed in breast tissue damage related to post mastectomy radiotherapy, breast augmentation, postmastectomy breast reconstruction and breast implant complications as capsular contracture.
In fact, in implant-based breast reconstructions, capsular contracture incidence has been reported ranging from 5% to 74% on behalf of several studies performed by implant manufacturers and prospective clinical studies with a considerable degree of follow-up (16-29). The capsular contracture is the development of a connective capsule around the prosthetic implant (Figure 1-2). The origin is unknown, but several hypotheses have been studied, such as inflammation, infection, hematoma, oozing of the silicone gel, the rupture of the prosthesis or an unspecific organism reaction to the implant. This capsule causes a variety of symptoms such as pain, induration, palpability and/or dislocation of the prosthesis itself. The capsular contracture can be measured in different grades (Baker classification) and may need surgical correction. Baker classification identifies three grades: grade I, described as soft capsule, normal breast appearance, no evidence of implant; grade II, with minimal capsule, palpable implant but not visible; grade III that causes moderate, firm breast, visible implant; grade IV characterized by severe, breast hard, breast distortion and discomfort or tenderness (30). Lipofilling is one of the most employed corrective techniques for capsular contracture, alone or with implant substitution.

*Figure 1-2: Capsular contracture*



Moreover, lipofilling has become successful because it could be performed under anaesthesia with sedation or just local anaesthesia as described by Coleman.
Lipofilling procedures help to remodel breast soft tissues, increasing skin thickness and tropism during the period of breast expansion (31-36). Furthermore, several lipostructures can be performed until a new breast is obtained, made only by the transferred fat. In addition, fat graft helps to reduce the number of future revisional surgery procedures, often needed after the first implant breast reconstruction for the development of capsular contracture.

Analysing the drawbacks of lipofilling, one of the most studied is the oncological potential of the grafted fat. Over the years, Authors have searched for a relationship between the placement of regenerated tissue in a tumour resection site and the possibility of promoting the tumour recurrence itself. In 2010, Rigotti and Sbarbati compared the incidence of local and regional recurrence of breast cancer between two contiguous time windows in a homogeneous population of 137 patients who underwent fat tissue transplant after modified radical mastectomy. The statistical comparison of disease-free survival curves shown no significant differences in relapse rate

between the two patient subgroups before and after the fat grafting (37). Petit in 2012 also concluded that lipofilling could be a safe procedure in breast cancer patients (38).
Although the reassuring conclusions of the studies conducted during these years, longer follow-up and further experiences from oncological series that study these findings will be received with great interest. In our Department of Plastic and Reconstructive Surgery only patients who are free of local breast disease are considered eligible for fat grafting, and all patients which will have surgery on the breast are required to provide recent radiological study of the breast, such as ultrasound examination and mammograms, no older than 12 months.

The main disadvantage of lipofilling is the unpredictable resorption rate of the graft. Fat graft survival is reported as ranging from 55% to 82% (39). As described by Largo et al., most of the volume loss occurs within the first 3 months after surgery (40). Therefore, aesthetic and functional results are not stable over time, leading to repetition of the surgery. As a consequence, methods to increase graft viability are needed and researched from plastic surgeons (41, 42). Other complications are cannula-related blunt injuries of blood vessels, nerves, muscles or fat embolism.

## 1.4 The Adipose Tissue

Adipose tissue is indeed a proper organ. Adipocytes express and secrete several endocrine hormones and protein factors such as leptin, adiponectin, TNF alpha and IL-6. Particularly interesting is that adipose tissue is capable of expression of adipokines involved in inflammation (list includes TNF$\alpha$, IL-1b, IL-6, IL-8, IL-10, TGF-$\beta$, NGF) and in the acute-phase response (plasminogen activator inhibitor-1, haptoglobin, serum amyloid A).

With liposuction, the surgeon collects both adipocytes and a heterogeneous cell population named stromal vascular fraction (SVF), a great source of mesenchymal stem cells (MSC). This population contains adult stem cells named adipose-derived stem cells (ADSCs), progenitor cells able to differentiate in multiple cell lineages (43, 44). In literature, several authors have demonstrated the trophic effect of stromal/stem cells on scars and irradiated zones, as well as the beneficial effects on angiogenesis and skin rejuvenation. These effects can probably be linked to growth factor secretion by adipose stromal cells (ASC) as well as their ability to differentiate into multiple lineages (45).

Several authors have studied how the choice of the donor site affects the quality of the fat itself in terms of better engraftment to the recipient site and lower reabsorption rate during the time. In 2010, Sbarbati investigated the characteristics of adipose tissue samples from various donor sites, evaluating their structural and ultrastructural characteristics and the concentration of adult stem cells (46). The study discovered three types of white adipose tissue (WAT):

- deposit WAT (dWAT), found essentially in large fatty depots in the abdominal area. In this tissue cells are tightly packed and the collagenic components are very poor. The deep portion appears more fibrous than the superficial one;
- structural WAT (sWAT), usually located in some areas in the limbs and in the hips. In the sWAT, the stroma is widely present and richly vascularized, and has adequate staminality;
- fibrous WAT (fWAT), which has noteworthy fibrous component and can be found in areas where a severe mechanic stress occurs.

The study of Sbarbati assumes that the sWAT could represent a privileged source for regenerative procedures based on autologous adipose tissues due to its microcirculation features.

The percentage of fat graft resorption rate and fat graft survival are quite unpredictable. These variables depend on three factors: mature adipocyte survival, differentiation of adipose-derived stem

cells (preadipocytes) in mature adipocytes and the presence of extracellular matrix, necessary for the two previously mentioned factors (9). The percentage of survival of a single fat graft varies between 55% and 82% and this number depicts the ratio between the amount of fat which has been integrated in the recipient breast tissue and the total amount of fat grafted during the surgery (40, 47). As previously described by Levine et al. (39), there is no standard test or imaging modality to determine fat viability during the time after lipofilling. Usually, ultrasound is employed as ordinarily procedure, because it is a simple technique, easy to perform and with no side effects for patients.
To increase fat graft viability, the overall homeostasis of the body should be improved. In literature, patient weight has been associated with breast reconstruction complications (48). Besides, weight loss has shown a decrease in inflammation (4, 6, 7).

## 1.5 The Diet as a Therapy

Several studies have observed the connection between fat resorption rate and presence of an inflammatory status (49-51). In 2017, Zhan et al investigated the influence of indomethacin, an anti-inflammatory pro-adipogenic drug, to the fat graft at the time of transplantation. In vitro, human adipose-derived stem cells were cultured in conditioned growth media supplemented with various doses of indomethacin to investigate adipogenesis and the expression of the adipogenic genes. In vivo, lipoaspirate mixed with stromal vascular fractions or indomethacin was injected into the dorsum of mice. Tissues were harvested at different time to evaluate histologic changes. It was observed that in vitro indomethacin increased up-regulation of adipogenic genes and activation of the peroxisome proliferator-activated receptor-$\gamma$ pathway. In vivo, the percentage volume of adipocytes in the indomethacin-assisted groups was higher than that in the lipoaspirate alone control group. However, indomethacin improved adipose volumes but had no effect on

vascularity. The authors concluded that indomethacin enhances the final volume retention of engrafted fat and explained this result in part by increased adipogenesis and possibly by the inhibition of inflammatory responses (49). Cervelli et al studied fat graft maintenance in two groups of patients which differed in body fat distribution and preoperative alterations of low-inflammatory markers, like neutrophil-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR). They found a minor fat graft resorption percentage in patients with high preoperative values of PLR and NLR compared with the group with normal preoperative NLR and PLR ratios (51).
A stimulating target is to be able to reach an anti-inflammatory status by the virtue of the diet.

In literature, several diets have been examined for this purpose. There is no agreement on which is the best diet that provides real anti-inflammatory results on both clinical and biochemical data. Promising results have been demonstrated by Longo et al, who have reported that periodic fasting in rodents and humans can reduce body fat, cut insulin levels and have a various range of effects on metabolic markers and risk factors, including blood pressure (52). Nevertheless, the diet studied by Longo is not available for all the patients, because are not provided free of charge and offered only to certain physicians and not to the Italian Public Health Service.

Among the other dietary regimens, once cleared of the ultimate cutting-the-edge trends, the Mediterranean Diet (MD) is the one that in different studies has shown promising results in terms of impact on the inflammatory status (53, 54). In 2016, the MOLI-SANI study addressed the relationship between the MD and platelet and leucocyte counts as emerging cellular biomarkers of low-grade inflammation, therefore the association of low- grade inflammation with a Mediterranean eating pattern (55).

As previously described from the Seven Countries Study, an International Cooperative Study on coronary heart disease

epidemiology promoted by the American physiologist Ancel Keys and the Italian Flaminio Fidanza, the MD is the traditional dietary pattern followed by Mediterranean populations in the early 1960s (56-62) and is characterized by the following (Figure 1-3):

1) abundant use of olive oil;
2) high consumption of plant foods: fruits, vegetables, legumes, cereals, nuts, and seeds;
3) moderate consumption of fish, seafood, fermented dairy products (yogurt and cheese), poultry, and eggs;
4) low consumption of red and processed meat and sweets;
5) frequent but moderate intake of wine (especially red wine) with meals: about 1-2 glasses per day for men and 1 glass per day for women (54, 63-69).

The Seven Country Study investigated the benefits of the MD on human health, comparing European rural cohorts (the Mediterranean cohorts Crete, Corfù, Crevalcore, Montegiorgio and Dalmatia) and the non–Mediterranean cohorts (East and West Finland, Slavonia and Velika Krsna) (54).

*Figure 1-3: The Mediterranean Diet (70)*

The PREDIMED (PREvenciòn con DIeta MEDiterrànea) study, a Spanish multi-center randomized trial of people at high risk for CVD, showed that a MD supplemented with nuts was able to reverse the metabolic syndrome more than a low-fat diet (54, 71). The metabolic syndrome is characterized by dyslipidemia and arterial hypertension, which are associated with increased risk of type II diabetes mellitus and cardiovascular diseases.
In addition, Authors have investigated the relationship among adherence to the MD and quality of life in breast cancer survivors in the ongoing multicentre randomized controlled trial "DEDiCa Study" (dietary modification, physical activity and vitamin D supplementation) in several Italian hospitals, determining that higher adherence to the MD provides higher physical functioning, better sleep, lower pain and generally higher well-being confirming findings in healthy subjects (72).

Overweight and obesity are linked to the occurrence of the metabolic syndrome as well. In fact, the adipose tissue is metabolically active and it is a proper endocrine organ, whose dysregulation causes a low-grade inflammatory state and ectopic fat depositions (54). The fat tissue produces inflammatory cytokines such as leptin, adiponectin, TNF-$\alpha$, IL-1, IL-6 and pro-coagulant substances (PAI-1) that are associated with a chronic low–grade inflammation. Also, the fat tissue can produce vasoactive factors and molecules promoting insulin resistance (IR): free fatty acids (FFA), resistin and retinol binding protein 4 (54, 73).
The apparent capacity of the traditional MD to reduce the risk of development and progression of cardiovascular disease, cancer and degenerative diseases has been attributed to nutraceutical effect of micronutrients and compounds with capacity antithrombotic, anticancer and antioxidant. Consequently, as stated by De Lorenzo, the healthy properties of the MD cannot be limited to any single nutrient, food or food component, but they have to be extended to the entire meal pattern and life-style (54).

Besides, the Mediterranean Diet is a sustainable diet in Italy, because all the nourishments are available on the local markets (74) and reflects environmental concerns and sustainability issues (70). The value of this diet lies in its ability to preserve the state of health and improve longevity, as United Nations Educational, Scientific and Cultural Organization (UNESCO) declared in 2010 (54).

## 2. Objective of the Study

The study aims to investigate the fat graft engraftment in patients who undergo breast reconstruction with implant and lipofilling after mastectomy.

### 2.1 Primary Endpoint

The primary endpoint is the rate of patients with successful fat graft engraftment, defined as the increase of at least 1 mm in the mean breast soft tissue thickness after lipofilling.

Co-primary endpoint is to evaluate if the adherence to the MD impacts on fat graft resorption rate, in terms of minor fat graft resorption. Patients are investigated in their dietetic habits and their adherence to the MD by means of a questionnaire that examines their usual daily and weekly consumptions.

### 2.2 Secondary Endpoint

The study collects the incidence of complications as fat necrosis, cysts, local recurrence, hematomas, seromas, bruising, prosthesis

exposure or infection, skin contour irregularities and fatty firmness and lumpiness, skin discoloration or swelling in both recipient and donor areas. Furthermore, questioning patients on their diet allows to have an insight on their food consumption, in particular to investigate their assumption of processed food.

## 3. Study Design

The study is a prospectic, single centre observational study.

## 4. Patients

Patients are women who experienced breast reconstruction with breast expander or prosthesis after mastectomy performed due to breast cancer. Informed consent has been obtained from all patients. The study is conducted according to the Declaration of Helsinki.

### 4.1 Inclusion Criteria

- Adult females who experienced mastectomy and undergo breast reconstruction with lipofilling on mastectomy skin flap. performed on prosthesis or breast expander.
- Skin thickness less than 1 cm in at least one breast quadrant
- Subjects of 18-70 years of age.
- Body mass index: 18.5-29.9 kg/m2 (normal weight to overweight, but not obese).
- Ability and willingness to provide written informed consent.
- If a patient is treated for bilateral breast reconstruction, she will be considered as a single case.

The patient's weight will be categorized using both body mass index (BMI), in kilograms per meter squared, and the World Health Organization Obesity Classification:

- Underweight (BMI <18.5 kg/m2).
- Normal weight (BMI 18.5 to 24.9 kg/m2).
- Overweight (BMI 25 to 29.9 kg/m2).
- Obese (BMI 30 to 40 kg/m2).

4.2 Exclusion Criteria

- Autologous reconstruction (DIEP flap, Latissimus Dorsi flap, etc).
- Drug dependency.
- Severe hypertension (systolic BP > 200 mm Hg and/or diastolic BP > 105 mm Hg).
- Women who are pregnant or nursing.
- Dietary reasons: special dietary requirements or significant food allergies incompatible with the study interventions, alcohol dependency defined as alcohol intake greater than two drinks per day for women.
- Tobacco use or genetic disorders will be not considered as exclusion criteria.

## 5. Investigation Plan

- Introduction of the study to patients and consent obtaining.
- Baseline characteristics are recorded in a database: age, smoking, comorbidities, unilateral versus bilateral disease and any chemotherapy, hormone treatment, or radiotherapy.
- Diet questionnaire compiling.
- Pre-operatory ultrasound ($T^0$) to measure breast skin thickness and representative preoperative photographs.

- Lipofilling surgical time.
- Post-operatory ultrasound after 3 months since the lipofilling surgery ($T^1$), along with diet questionnaire compiling and representative preoperative photographs. The gain in the soft tissue thickness after one lipofilling is evaluated measuring the depth of fat tissue from skin to pectoralis muscle fascia. Data will be compared to the pre-operatory measure.
- Fat graft reabsorption rate measure.
- Patients grouping according to MD adherence (Figure 5-1), evaluated using the previously validated 14-item questionnaire for the assessment of PREvención con DIeta MEDiterránea (PREDIMED) (75).
- Statistical analysis.
- Results.

*Figure 5-1: Grouping of the patients*

## 6. Methods

### 6.1 The Questionnaire

The adherence to the MD has been evaluated using the previously validated 14-item questionnaire for the assessment of PREvención con DIeta MEDiterránea (PREDIMED) (76). This survey is simple to understand by patients compared to other forms available in literature and has already been translated in Italian as previously applied in different studies (36, 53, 76-79).

The questionnaire consists of a 14-point MD score (80) (Table 6-1), administered two times:

- before the surgical procedure. The questionnaire has been provided to patients during the medical survey.
- when patients have their ultrasound follow up, from three months after the surgical procedure.

The double check helps to understand if there have been any changes in the dietary regimen.

Briefly, for each item, scores 1 and 0 are assigned; PREDIMED score has been calculated as follows:

- 0–5 points of the 14-item questionnaire, lowest adherence;
- score 6–9 points of the 14-item questionnaire, average adherence;
- score ≥ 10 points of the 14-item questionnaire, highest adherence.

*Table 6-1: The Questionnaire*

| **Table 1** | |
|---|---|
| **QUESTIONS** | Criteria for 1 point |
| **1. Do you use olive oil as main culinary fat?** | Yes |
| **2. How much olive oil do you consume in a given day (including oil used for frying, salads, out-of-house meals, etc.)?** | ≥4 tbsp |
| **3. How many vegetable servings do you consume per day? (1 serving: 200 g [consider side dishes as half a serving])** | ≥2 (≥1 portion raw or as a salad) |
| **4. How many fruit units (including natural fruit juices) do you consume per day?** | ≥3 |
| **5. How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per day? (1 serving: 100–150 g)** | <1 |
| **6. How many servings of butter, margarine, or cream do you consume per day? (1 serving: 12 g)** | <1 |
| **7. How many sweet or carbonated beverages do you drink per day?** | <1 |
| **8. How much wine do you drink per week?** | ≥7 glasses |
| **9. How many servings of legumes do you consume per week? (1 serving: 150 g)** | ≥3 |
| **10. How many servings of fish or shellfish do you consume per week? (1 serving 100–150 g of fish or 4–5 units or 200 g of shellfish)** | ≥3 |
| **11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits, or custard?** | <3 |
| **12. How many servings of nuts (including peanuts) do you consume per week? (1 serving 30 g)** | ≥3 |
| **13. Do you preferentially consume chicken, turkey, or rabbit meat instead of veal, pork, hamburger, or sausage?** | Yes |
| **14. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic and simmered with olive oil)?** | ≥2 |

The study compares the gain in breast skin thickness in patients grouped according to the three categories of adherence to the Mediterranean diet:

- 0–5, the lowest adherence;
- score 6–9, average adherence;
- score ≥ 10, the highest adherence.

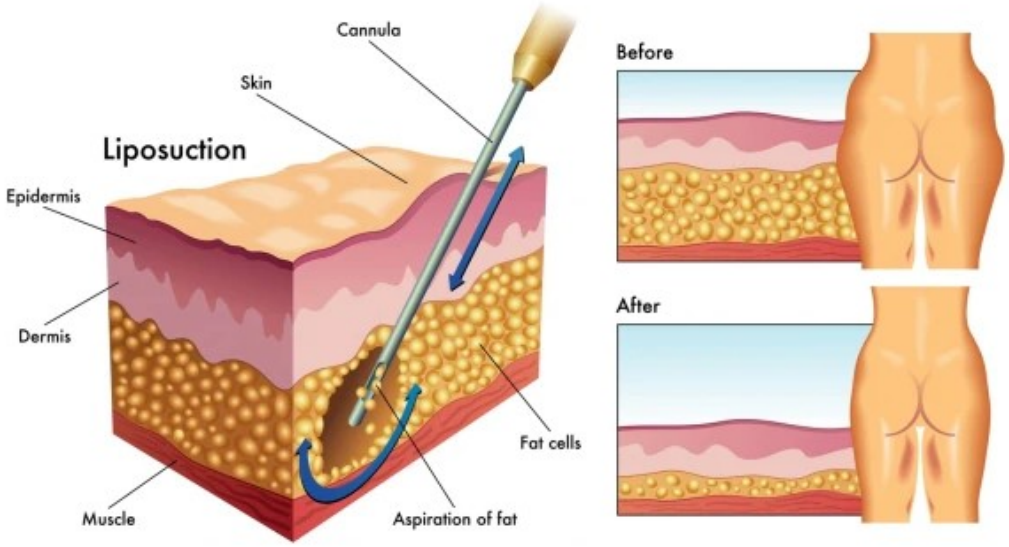
## 6.2 The Surgical Technique

The procedure of autologous fat grafting requires several steps (47). All procedures are performed under general anesthesia. Antibiotic prophylaxis is routinely administered (intraoperative cefazoline 2 g).

Depending on the patients' demand and fat availability, donor areas usually are the abdomen, hips, and inner and/or outer thighs. Harvesting sites are accessed through incisions placed usually in the creases, previous scars, stretch marks or hirsute areas (14).

First, sites are infiltrated with combined saline solution and diluted adrenaline solution (1 cc of adrenaline in 1 litre of saline) (11) (Figure 6-1).

*Figure 6-1: The liposuction*



Then, the fat tissue is suctioned through the incisions made for infiltration of the anesthetic solution using a cannula in a sterile closed system created by connecting the liposuction cannula to a sterile drain bottle, which is connected to the liposuction machine on the other end. Fat grafting should be efficient and minimally traumatic to the grafted tissue both during the harvesting and the placement phases. The cannula employed is a two-holed one with a blunted tip, shaped like a bucket handle, to encourage harvesting of small parcels of fat (14).

After aspiration, the fat is transferred in syringes which are closed with a plug and stored vertically in a metal grill. The syringes are stored on a table to allow the fatty tissue to separate from the liquid component (Figure 6-2). The aqueous portion can be expressed by pressing on the syringe (45). After a while, the syringes will show three different layers which differ in density. The upper level or the less dense level is composed primarily of oil, presumably from ruptured cells. The middle portion is composed primarily of potentially viable parcels of tissue, with more oil present in the upper portion and denser connected tissue present in the lower portion. The lowest level is the densest layer and is composed primarily of blood, water, and anesthetic. Discarding the oily part and the densest level, this procedure obtains purified fat tissue, preserving the integrity of the adipocytes but separating the fluid fat portion from the serous bloody part (Figure 6-3).

During fat harvesting, liposuction incisions are closed using 5-0 or 6-0 nylon interrupted sutures.

*Figure 6-2: Processing of the liposuctioned fat*

*Figure 6-3: The purified fat tissue*

Therefore, the purified body fat is placed in 3 cc syringes connected to cannulas and reinserted into the breast (Figure 6-4). Exposure of the fatty tissue to open air is avoided whenever possible, because histologic studies have demonstrated cytoplasm lysis of up to 50% of the cells exposed to air for 15 minutes (14).

In the end, the patient is checked for any asymmetry and deformities, that are meticulously corrected.

*Figure 6-4: Grafting of the fat*

During fat grafting, the purified fat is released in the subcutaneous tissue with a retrograde technique while withdrawing the cannula and placed along linear deposits. It is implanted in multiple tunnels to ensure a better contact with vascularized bed, and within each tunnel, it is released in small aliquot to increase the viability of central adipocyte cells and also to ameliorate fat particle integration (47).
Fat graft is operated depending on the thickness of the mastectomy's skin flap (34, 81). The amount of fat grafted is recorded in the patient's notes.

Risk are among the common ones described during breast reconstruction surgery with lipofilling.
Risks and complications comprise:

- Surgical anaesthesia risk and complications: both local and general anaesthesia involve risk. There is the possibility of complications, injury and even death from all forms of surgical anaesthesia or sedation.

- Surgical procedure risk and complications. There is the possibility of complications, injury and even death during any surgical procedure:
  - Haematomas
  - Seromas
  - Bruising
  - Swelling
  - Pneumothorax
  - Prosthesis exposure or infection
  - Skin contour irregularities and fatty firmness and lumpiness
  - Skin discoloration or swelling
  - Major wound separation
  - Delayed healing, scarring and poor wound healing
  - Fat and skin necrosis with tissue lost (death of transferred fat tissue)
  - Cysts may also form at the site of the transferred fat
  - Embolism
  - Damage to deeper structures including blood vessels, muscles
  - Nerves injury: motor and sensory nerve injury, weakness or loss of movements may occur. Nerve injuries may cause temporary or permanent loss of movement and feeling. Such injuries may improve over time. Painful nerve scarring is rare, but can occur
  - Bleeding or infections occur: additional treatment including antibiotics, hospitalization, or additional surgery may be necessary.
- Plastic surgery risk and complications: unsatisfactory results that may lead to additional surgery.
  - Bruising
  - Swelling
  - Skin contour irregularities and fatty firmness and lumpiness
  - Skin discoloration or swelling in both recipient and donor areas.

These issues are monitored by means of the ultrasound and during the follow-up.

## 6.3 Patients Follow-up

Patients have been followed-up in the outpatient clinic to assess fat graft survival and potential complications as usual clinical practice. Follow-up visits entail:

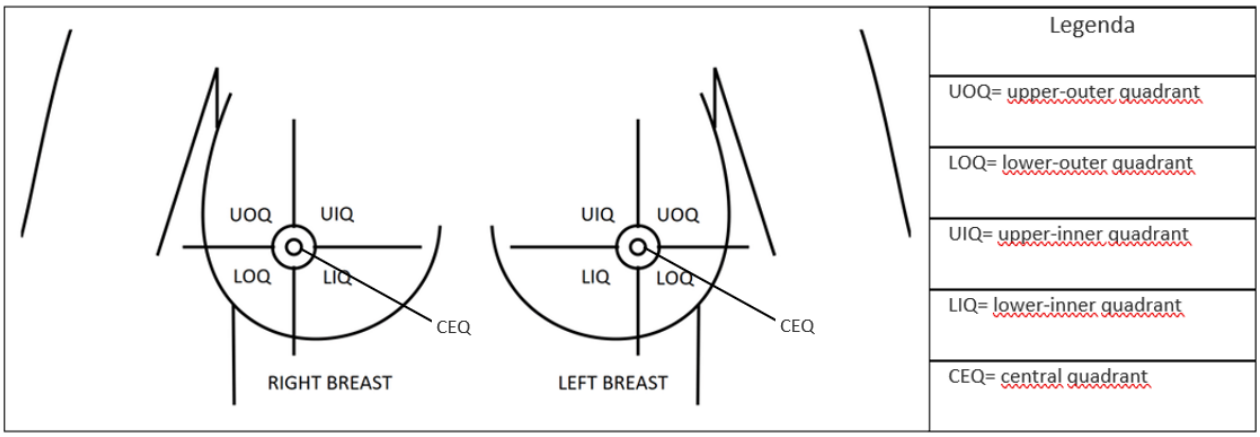
- A physical examination.
- Postoperative photographs of the mammary region, according to standard projections.
- Breast ultrasound.

The follow-up protocol for this study is current standard of care for the patients of our Department of Plastic and Reconstructive Surgery.

## 6.4 Ultrasound Evaluation

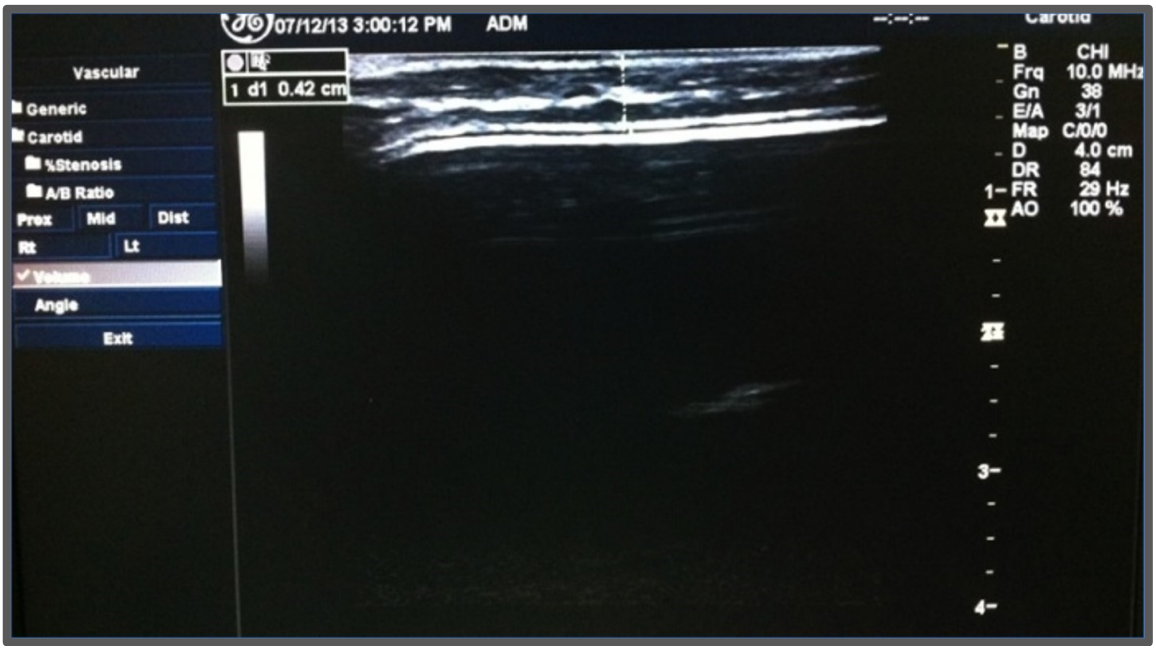
Breast soft tissue thickness is calculated by means of an ultrasound evaluation of the depth of fat tissue from skin to pectoralis muscle fascia. Ultrasound evaluation is operated for every patient in every breast quadrant of the treated breast: upper outer quadrant, upper inner quadrant, lower outer quadrant, lower inner quadrant, central quadrant (areola-nipple) (Figure 6-5), as routine clinical practice (82-85). The fat thickness is measured at the middle point of the quadrant. If the patient has no nipple, the maximal projection of breast mound substitutes the point of nipple.

*Figure 6-5: Breast quadrants*




The ultrasound is performed pre-operatory ($T^0$) and from three months after surgery ($T^1$). At $T^1$, the ultrasound measures any variation in mastectomy skin flap thickness after lipofilling in terms of space from skin to pectoralis muscle fascia (Figure 6-6). Then, data are compared.

*Figure 6-6: Ultrasound evaluation of breast soft skin thickness*



6.5 Fat Reabsorption Rate

As successful fat graft engraftment the study defines the increase of at least 1 mm in the mean breast soft tissue thickness after one lipofilling.

## 7. Statistics

7.1 Sample size

**Primary endpoint**
Considering that there are no published data on the proportion of women with low lipofilling resorption rate, we assume that this proportion is 50% (i.e., the proportion that maximizes the standard error). Consequently, a sample size of 93 patients is sufficient to estimate such a proportion with a precision of 10%, two-sided confidence interval 95%.

**Co-primary endpoint**
For sample size calculation the study assumes that the proportion of women with high lipofilling resorption rate (cases) is 50%; controls are the patients with low lipofilling resorption rate. Assuming that low adherence to Mediterranean diet is 25% in cases (high lipofilling resorption) and 50% in controls, a total of 98 patients (49 cases and 49 controls) is sufficient to estimate an odds ratio of 0.33 (protective effect of Mediterranean diet), given the a priori probabilities alpha=0.05 and beta=0.20.

7.2 Data Analysis

All the patients enrolled and with complete data are included in the analyses.
Since this is an observational study, descriptive summaries are presented for all the patients and for subgroups of patients.
The rate of women with fat graft engraftment is calculated and described with corresponding confidence interval.
Paired t-test or Wilcoxon matched-pairs signed-rank are employed to evaluate the mean gain in soft tissue thickness in the breasts, as appropriate.
The t-test or Wilcoxon's rank-sum test are employed to compare the fat gain in the two independent groups of patients (MD-adherent and not MD-adherent), as appropriate.
Comparison among categorical variables is performed using chi-squared test or Fisher exact test, as appropriate.
For continuous variables, normality is assessed using Shapiro-Wilk test.

## 8. Results

The pandemic related to SARS-CoV-2 infection caused in Italy over 71.000 deaths and one million infections from January 2020 to December 2020 (86). As a result, Italian Ministry of Health chose to stop non-oncological surgeries and controls during the peak of the pandemic to preserve hospital workforce, often differently employed in COVID-19 management, and free the operating theatres which have been converted to intensive care units. Udine Plastic and Reconstructive Surgery Department has been affected by the restrictions, and only post-traumatic patients and oncologic cases have been treated, with a drop of breast reconstructive surgeries and post-operatory controls.

This has affected the study, which collected a total of 11 patients with complete pre-operatory and post-operatory controls enrolled in the study (Table 8-1 and 8-2).
Three patients were excluded from the study: one patient developed infection (the only complication observed) and had her expander to be removed, two patients refused to come to the post-operatory control due to fear of COVID-19.

*Table 8-1: Results I*

| ID | GRAFT cc | MD ADHERENCE | T0 SP mean (mm) | T1 SP mean (mm) | PO CONTROL months |
|---|---|---|---|---|---|
| 1 | 115 | 12 | 10,7 | 14,3 | 7 |
| 2 | 130 | 11 | 6,4 | 6,7 | 7 |
| 3 | 160 | 8 | 6,0 | 6,3 | 8 |
| 4 | 100 | 9 | 4,3 | 4,9 | 8 |
| 5 | 140 | 10 | 4,8 | 6,0 | 4 |
| 6 | 120 | 7 | 5,0 | 5,5 | 4 |
| 7 | 110 | 7 | 2,8 | 3,4 | 4 |
| 8 | 100 | 8 | 3,4 | 3,9 | 3 |
| 9 | 100 | 7 | 6,4 | 7,0 | 3 |
| 10 | 150 | 6 | 5,8 | 6,3 | 5 |
| 11 | 90 | 7 | 3,2 | 3,9 | 6 |

*Table 8-2: Results II*

| ID | AGE | PREVIOUS LIPOFILLINGS | COMORBIDITIES | COMPLICATIONS | SMOKE HABIT | BMI | RT PRE | CHT PRE | HORMONE TP | BREAST |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 55 | 2 | 0 | 0 | 0 | 24 | 0 | 0 | 1 | L |
| 2 | 56 | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 1 | R |
| 3 | 73 | 0 | 1 | 0 | 0 | 26 | 0 | 0 | 0 | L |
| 4 | 55 | 0 | 0 | 0 | 0 | 21 | 1 | 0 | 1 | R |
| 5 | 50 | 0 | 1 | 0 | 0 | 24 | 1 | 0 | 1 | R |
| 6 | 44 | 0 | 1 | 0 | 0 | 22 | 0 | 0 | 0 | R |
| 7 | 47 | 0 | 1 | 0 | 0 | 23 | 1 | 1 | 1 | R |
| 8 | 40 | 0 | 0 | 0 | 1 | 24 | 0 | 1 | 1 | R |
| 9 | 53 | 0 | 0 | 0 | 1 | 23 | 0 | 0 | 0 | R |
| 10 | 32 | 0 | 0 | 0 | 1 | 21 | 0 | 1 | 0 | L |
| 11 | 31 | 0 | 0 | 0 | 0 | 19 | 0 | 1 | 1 | L |

There were no patients with low adherence to the Mediterranean Diet in Friuli Venezia Giulia. Eight patients (73%) have an average adherence to the MD, three patients (27%) have a high adherence to the MD. All the patients expressed their same adherence to the MD

during the questionnaire compiling at both the pre-operatory time and the post-operatory time.
Analysing the answers to the questionnaire, few patients reported to eat legumes, dried fruit or nuts and wine consumption.

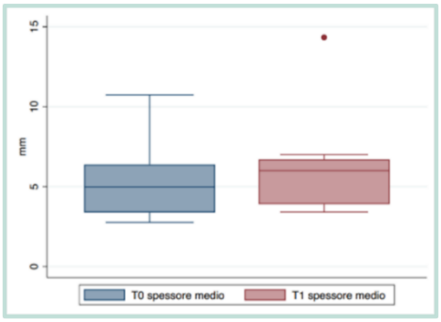
The mean age of patients is of 49 years, SD 12 y. Three patients are active smokers (27%), four patients have comorbidities (36%).
The mean BMI of patients is 22, SD 1.90 (one overweight patient).
One patient had previous lipofillings (2).
The average amount of fat grafted is 119 cc, SD 22.85, median 115 cc, range [90-160 cc].
Four patients had pre-operatory chemotherapy (36%), 3 patients had pre-operatory radiotherapy (27%), 7 patients with current hormonotherapy (64%).

The mean time of the post-operatory control is of 5 months, SD 1.89, median 5 months, range [3-8 months]. The mean pre-operatory soft tissue thickness is of 5.32 mm, SD 2.20, median 4.98 mm, range [2.76-10.74 mm].

The mean post-operatory soft tissue thickness is of 6.20 mm, SD 2.96, median 6.00 mm, range [3.40-14.32 mm]. The larger post-operatory thickness was measured in the patient who had already undergone the lipofilling surgery. The post-operatory thickness has not shown normal distribution (Figure 8-1).

The gain in breast soft tissue thickness from T0 to T1 is statistically significative: 0.062, range [0.032-3.59 mm] (p= 0.00001).

*Figure 8-1: The post-operatory thickness*



Two patients have at least 1 mm of gain in soft tissue thickness. The study did not find statistically significative association between Mediterranean Diet adherence and gain ≥1 mm in thickness (p= 0.055).

Moreover, no statistically significative association among gain in breast soft tissue thickness and previous lipofillings, comorbidities, previous RT/CHT, smoke habit, hormonotherapy was found.
No association between gain in soft tissue thickness and amount of fat grafted was found.

Figures 8-2 and 8-3 depict two cases of breast reconstruction with prosthesis (figure 8-2) and with breast expander (figure 8-3).

*Figure 8-2: Clinical case, breast reconstruction with prosthesis and previous radiotherapy. Lipostructure alone.*

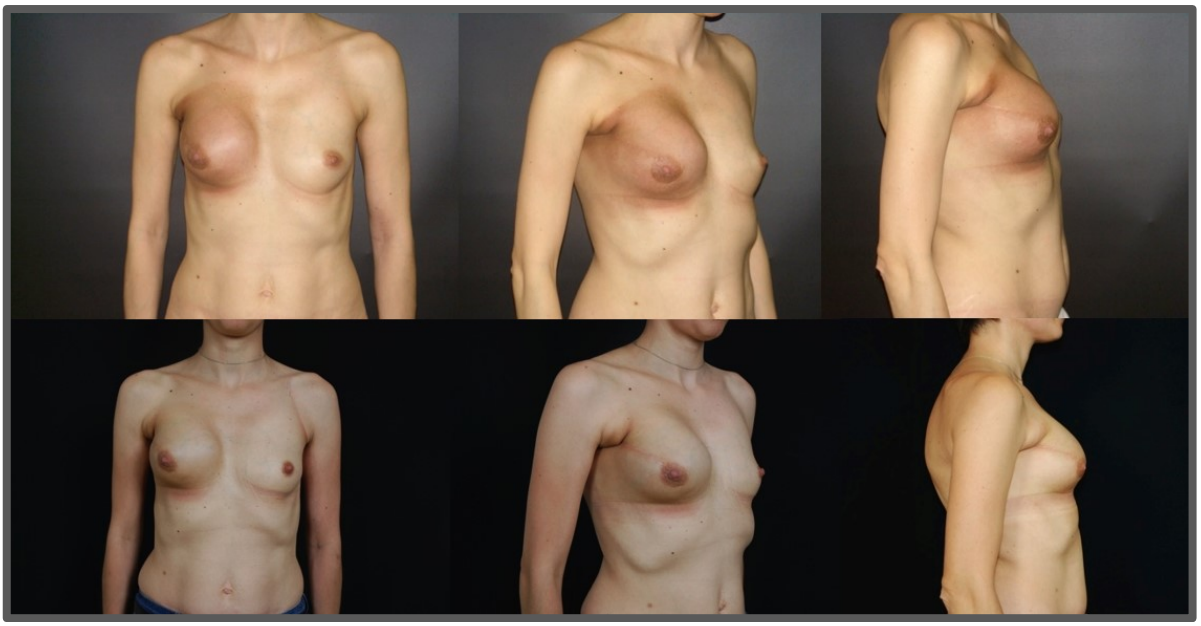
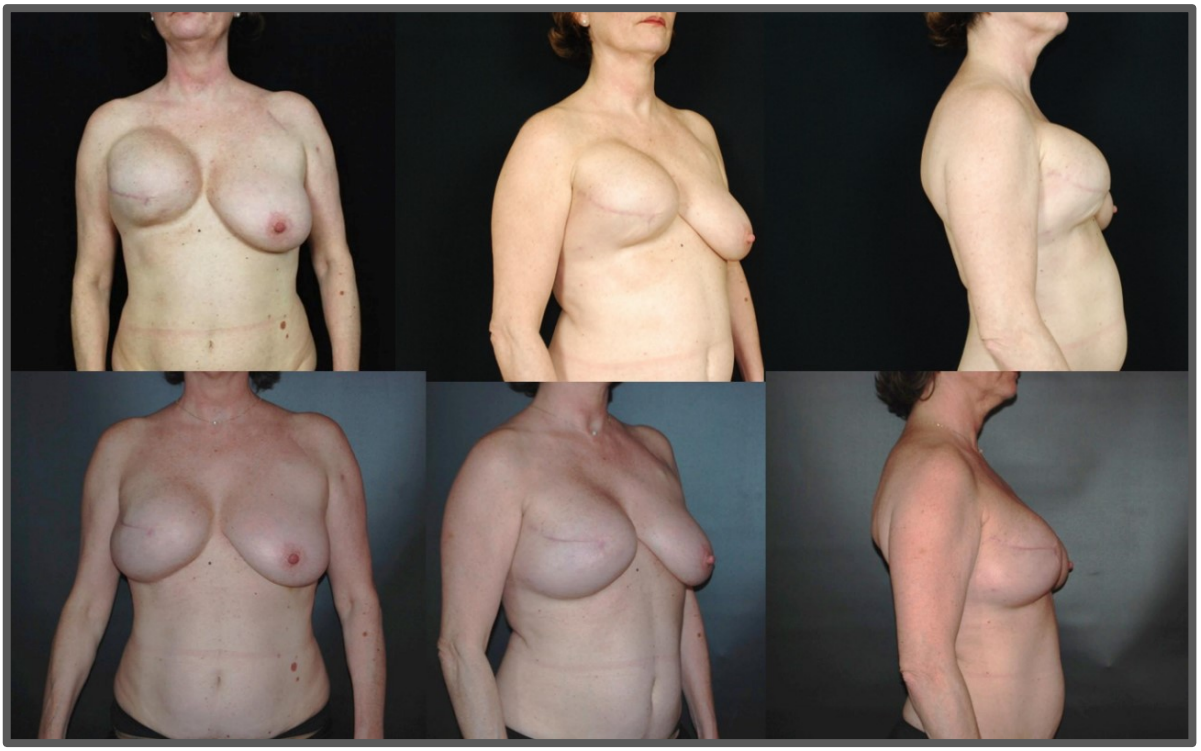


*Figure 8-3: Clinical case, breast reconstruction with breast expander. Expander substitution with prosthesis and lipofilling*



## 9. Discussion

As a result of COVID-19 pandemic, the study has collected really few patients to satisfy the sample size target, and this has impacted the possibility to find statistically significative association.
In particular, considering the sample of 11 patients, assuming that the proportion of women with low lipofilling resorption rate is 50% (i.e., the proportion that maximises the standard error), the study can estimate such proportion with a precision of 30%, two-sided confidence interval 95%.
Besides, the small number of patients did not allow the proper investigation of the role of previous RT, previous lipofillings or smoke habit in the reabsorption of the fat.

The COVID-19 pandemic produced a wide range of time of the post-operatory controls and ultrasounds, from 3 to 8 months, with a mean time of the post-operatory control of 5 months. This led to view the grafted fat in different times of reabsorption and did not allow to study patients in proper scheduled times. As described by Dong Won Lee et al, a strict protocol with pre-operatory measures and post-operatory follow-up within 1 month and after 6 months from the procedure, including physical examination and ultrasonography, allows to employ formulas to calculate the exact ratio of the fat reabsorption rate (82).
The difficulties comprised also the availability of other techniques for fat graft retention investigation, as CT, MR or 3D photography (85, 87), and were extended to the difficult compliance of the patients. In fact, often women reported to be scared of becoming infected with SARS-CoV-2 if coming to the hospital for the visits.

The patients had a gain in breast soft tissue thickness: the mean pre-operatory record was 5.32 mm whereas the mean post-operatory one was of 6.20 mm, and the gain in breast soft tissue thickness from T0 to T1 is statistically significative (0.062, p= 0.00001). This result is in line with the literature on breast reconstruction with lipofilling

(39, 88). The larger post-operatory thickness was measured in the patient who had already undergone the lipofilling surgery, although the data have not been sufficiently numerous to find statistically significative association as previously described from other Authors (89).

Due to the few participants, the study did not find statistically significative association between Mediterranean Diet adherence and gain ≥1 mm in thickness ($p= 0.055$). Furthermore, there was no possibility to investigate the influence of previous lipofillings, comorbidities, previous RT/CHT, smoke habit, hormonotherapy and amount of grafted fat as conducted by other Authors (45, 89).

However, the study permits to observe that there is a good adherence to the Mediterranean Diet in Friuli Venezia Giulia. In fact, all the patients of the study reported to feed themselves according to the MD: 73% of patients described an average adherence to the MD and 27% of women have a high adherence to the MD. Moreover, several patients described changes in their dietary habits after the diagnosis of cancer, with active effort to do their best. This result is in line with the literature: a survey performed by Zhang et al among breast cancer survivals depicted at least 1 positive behaviour for improving nutrition and physical activity after cancer diagnosis or treatment (90).

Patients reported to eat few legumes, dried fruit or nuts and described low wine consumption. Answering the questionnaire, women could have been influenced by the fact that people feel under exam when investigated on their diet. As a matter of fact, legumes are present in typical Friulan dishes, as *pasta e fagioli* or *minestrone*. In addition, wine consumption is quite widespread in Friuli Venezia Giulia. In the Author's opinion there is no point to think that the answers were false, because women of the sample size are healthy and in a good shape, with just one patient overweight. Besides, other studies encourage to support a widespread habit at the Mediterranean Diet in Friuli Venezia Giulia (72, 91).

## 10. Conclusions

Lipofilling produces a gain in breast soft tissue thickness.

Following the diagnosis of cancer, Friulan women pay attention to their diet and feed themselves according to the Mediterranean Diet, with active changes in their lifestyle.

On one hand, the COVID-19 pandemic produced a lack in enrollment of enough patients to investigate if there is a correlation between fat graft resorption rate and Mediterranean Diet adherence in Patients who undergo the lipofilling procedure.

On the other hand, the study helped to understand the dietary habits of the patients.

Future studies could lead to guidelines for the management of cancer survival patients who undergo breast reconstruction with lipofilling, in order to suggest them a way to improve both their breast reconstruction results and their quality of life.
Moreover, results will help to investigate the association among fat graft survival rate and the influence of single elements of the diet.
Furthermore, other studies could characterize the ADSCs of patients with low lipofilling resorption rate or analyze the oxidative and nitrosative stress in the fat graft.

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